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THE EFFECT OF SODIUM-FREE AND POTASSIUM-FREE SOLUTIONS, IONIC CURRENT INHIBITORS AND OUABAIN ON ELECTROPHYSIOLOGICAL PROPERTIES OF SMOOTH MUSCLE OF GUINEA-PIG URETER

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SUMMARY

1. The effects of Na-free and K-free solutions, tetraethyl ammonium (TEA), Mn^{2+}, verapamil and ouabain on the electrophysiological properties of the smooth muscle cells of guinea-pig ureter have been studied, using the double sucrose-gap method.

2. TEA (5 mM) increased the amplitude and duration of both the initial spike component and the subsequent plateau of the action potential. The repetitive spike discharge on the plateau was abolished. The amplitude and duration of the phasic contraction was increased. The threshold for excitation was lowered while the resting potential and membrane resistance were unaffected.

3. In Na-free solution the duration of the action potential decreased, mainly due to the suppression of the plateau. A similar effect was produced by exposure to K-free solution and also by ouabain.

4. Mn^{2+} (2 mM) suppressed the spike component and raised the threshold for excitation. The amplitude of the remaining part of the action potential was markedly increased but the contraction was rapidly abolished. The resting potential and membrane resistance were unchanged.

When Mn^{2+} was added to Na-free solution it produced an increase in the amplitude and duration of the remaining part of the action potential but the phasic contraction was abolished.

5. Verapamil did not specifically block the fast component of the action potential but initially increased the amplitude of the spike and shortened the plateau. Subsequently, both the action potential and the phasic contraction became smaller.

6. The observations indicate that the phasic contractions are triggered

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by the initial spike component of the action potential, whereas the plateau is associated with the amplitude and particularly the duration of the contraction.

INTRODUCTION

Investigations of the effects produced by changing the external Na^+ and Ca^{2+} concentration on the action potential (Kobayashi, 1965, 1969; Kuriyama & Tomita, 1970; Kochemasova, 1971) and on the transmembrane ionic currents of the ureter muscle cells (Shuba & Bury, 1971; Bury, 1973; Bury & Shuba, 1974) suggest that the initial fast component of the action potential is due to Ca^{2+}-influx, whereas the subsequent slow component, the plateau, is due to Na^+-influx. However, the evidence for this is as yet incomplete since the mechanism of action of some other ions and specific drugs on the ureter have not been fully studied. This was the aim of the present work. In addition, the relationship between the different components of the action potential and the contraction of the ureter muscle cells has been investigated.

METHODS

The experiments were performed on isolated pieces of the longitudinal muscle layer from the middle part of the guinea-pig ureter by means of the double sucrose-gap method as described by Bülbring & Tomita (1969) which is essentially the same as that of Berger (1963), Shuba (1963) and Artemenko & Shuba (1964). The length of ureter under study, i.e. the central part between the two sucrose-gap regions, was 1 mm.

Two normal control solutions were used containing (mM):

	NaCl	KCl	$CaCl_2$	$NaHCO_3$	$MgCl_2$	NaH_2PO_4	Glucose	O_2/CO_2%
(1) Modified Krebs solution	120·4	5·9	2·5	15·4	1·2	1·2	11·5	97/3
(2) Modified Locke (low bicarbonate) solution	134·0	5·9	2·5	5·9	—	—	11·5	100/0

The temperature was kept at 34–35 °C. Low bicarbonate solution (pH $7·7 \pm 0·2$) was used for experiments in which Mn^{2+} was added, or Na removed. Na^+-free solution was prepared by replacing NaCl with the osmotically equivalent amount of either Tris Cl or sucrose and replacing $NaHCO_3$ with $KHCO_3$, KCl being omitted. K^+-free solution was prepared by substitution of KCl with an equivalent amount of glucose.

Tetraethyl ammonium (TEA) and Mn^{2+} chlorides, verapamil (Isoptin, Knoll) and ouabain (Strophanthin G, British Drug Houses) were dissolved in Krebs or Locke solution in the appropriate concentration.

Anelectrotonic potentials were evoked by rectangular current pulses of the order of 10^{-7} A and 2–3 sec duration. Catelectrotonic potentials, evoked by currents of similar intensity and duration, reached or exceeded firing threshold. Action potentials were therefore usually evoked by depolarizing currents of shorter duration (30–50 msec) to aoid the possible influence of prolonged depolarization on the shape and amplitude of the action potential. Electrical and mechanical activity were recorded with a Clevite Brush Mark 220 two channel pen recorder.

RESULTS

Under normal conditions, the action potential of ureter muscle cells, evoked by threshold and stronger depolarizing current, has an initial fast component consisting of repeated and gradually decaying spikes and a subsequent slow component, the so-called plateau (Fig. 1*A*). The action potential is accompanied by a brief contraction lasting 2–3 sec.

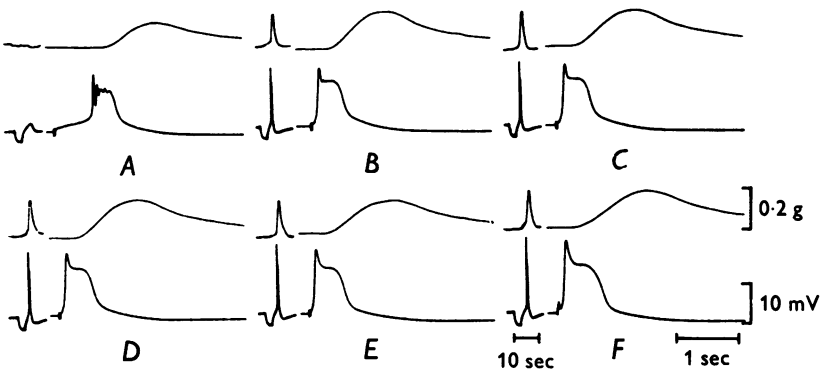

Fig. 1. Effect of TEA on ureter muscle. In this and subsequent Figures (except Fig. 2*A*) the upper trace shows the mechanical and the lower trace the electrical response to a constant current pulse (double sucrose-gap method). In each record, the response to the anodal current pulse is recorded on a slow time base (calibration, 10 sec) and is followed by the response to the cathodal current pulse on a fast time base (1 sec): *A*, in normal Krebs solution; *B–F*, during exposure to TEA (5 mM) for 1, 3, 5, 10 and 25 min.

Effect of TEA

The addition of 5 mM TEA to normal Krebs (or Locke) solution lowered the threshold of excitation, increased the amplitude and duration of the first spike and also of the plateau; it suppressed the repetitive discharge. At the same time the amplitude and duration of contraction was increased. All these changes occurred within the first minute of the TEA action (Fig. 1*B*). In addition, the latency of the action potential was markedly decreased and an action potential appeared upon termination of a hyperpolarizing current pulse (off-spike). All the effects mentioned became gradually larger as the exposure to TEA continued, though the resting potential and the membrane resistance, judging by the magnitude of the electrotonic potential, were not substantially changed (Fig. 1*C–F*). The changes of the action potential and the contraction reached their maximum after 10–15 min. By this time, the increase of the amplitude of the fast and slow components of the action potential was considerable. The duration

of the contraction increased to a greater extent than its amplitude. A similar relation was also observed between amplitude and duration of the action potential after prolonged exposure to TEA.

The effect of Na-free solution

In Fig. 2*A* (*a*), recorded under normal conditions, the action potential in response to a depolarizing current showed a clear initial fast and subsequent slow component. Anode-break excitation was absent. Within the

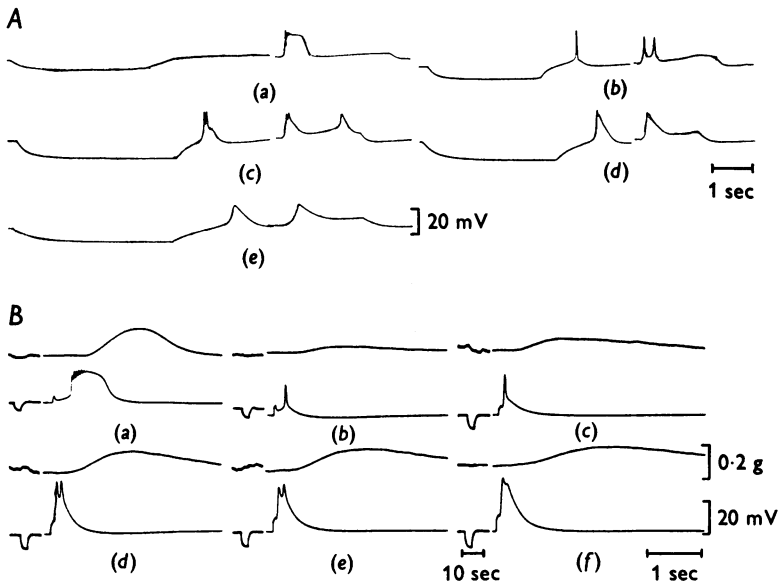

Fig. 2. *A*, the effect of Na-free solution (sucrose substitution) on electrical responses to hyperpolarizing and depolarizing current pulses, recorded on same time base (1 sec): (*a*) in normal Locke solution; (*b–e*) in Na-free solution for 0·25, 15, 30 and 60 min respectively. No mechanical record. *B*, the effect of Na-removal (Tris substitution): (*a*) in normal Locke solution; (*b–f*) in Na-free solution for 1·2, 30, 100, 120 and 140 min respectively. In (*b*) and (*c*) the current intensity was increased to 1·25-times, and in (*d–f*) to 1·5-times that used in (*a*).

first minutes of exposure to Na-free solution a hyperpolarization was observed, the electrotonic potential increased about twofold and an off-spike appeared, as a simple spike without plateau (Fig. 2*A* (*b*)). At the same time, the evoked action potential had a similar shape (Fig. 2*A* (*b*)). Within the next few minutes after Na-removal the membrane gradually re-polarized and the duration of the action potential increased, probably due to gradual superposition of two spikes (Fig. 2*A* (*c–e*)).

Replacement of Na^+ ions with 2-amino-2-hydroxymethylpropane-1,3

diol (Tris) brought about similar electrophysiological changes to those seen in Na-free sucrose solution except that no off-spikes were elicited by breaking anodal current pulses (Fig. 2*B*). It is interesting to note that, although the duration of the action potential was reduced, the contractions were considerably prolonged while their amplitude was slightly decreased. During continued exposure to Na-free Tris-solution, the duration of the action potential became longer and double spikes appeared (as in Fig. 2*A*). It is difficult to say whether this effect is the result of superposition of two action potentials or of increased duration of the individual action potentials.

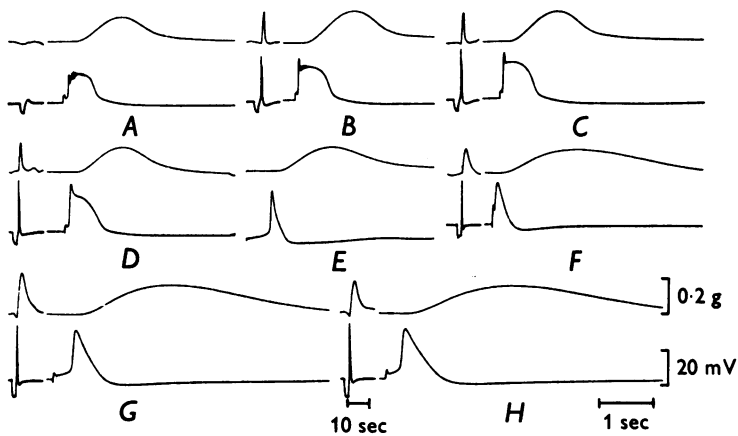

Fig. 3. Effect of TEA followed by Na-removal: *A*, in normal Locke solution; *B*, *C* and *D*, 0·5, 2 and 30 min in TEA (5 mM); *E–H*, 2·5, 4, 10 and 15 min after Na removal (replacement with sucrose) in the presence of TEA. A spontaneous action potential and contraction were recorded in *E*. Current intensity, relative to that applied in *A–C*, was reduced to 0·6 in *D*, 0·35 in *G* and 0·27 in *H*.

This question could be partly clarified by Na^+ removal in the presence of TEA. It has already been noted, that TEA suppressed the repetitive spike discharge during the action potential. In the experiment shown in Fig. 3, it can be seen that TEA increased the amplitude and duration of the first spike, while additional spikes were abolished (Fig. 3*A–D*). During 2–4 min after Na withdrawal the duration of the action potential decreased, mainly as a result of the reduced plateau (Fig. 3*E*, *F*). Simultaneously the amplitude of contraction also decreased though the duration increased. However, subsequently the duration of the action potential became gradually longer, probably as a result of delayed repolarization (Fig. 3*G*, *H*). At the same time the amplitude and especially the duration of the contraction also increased further.

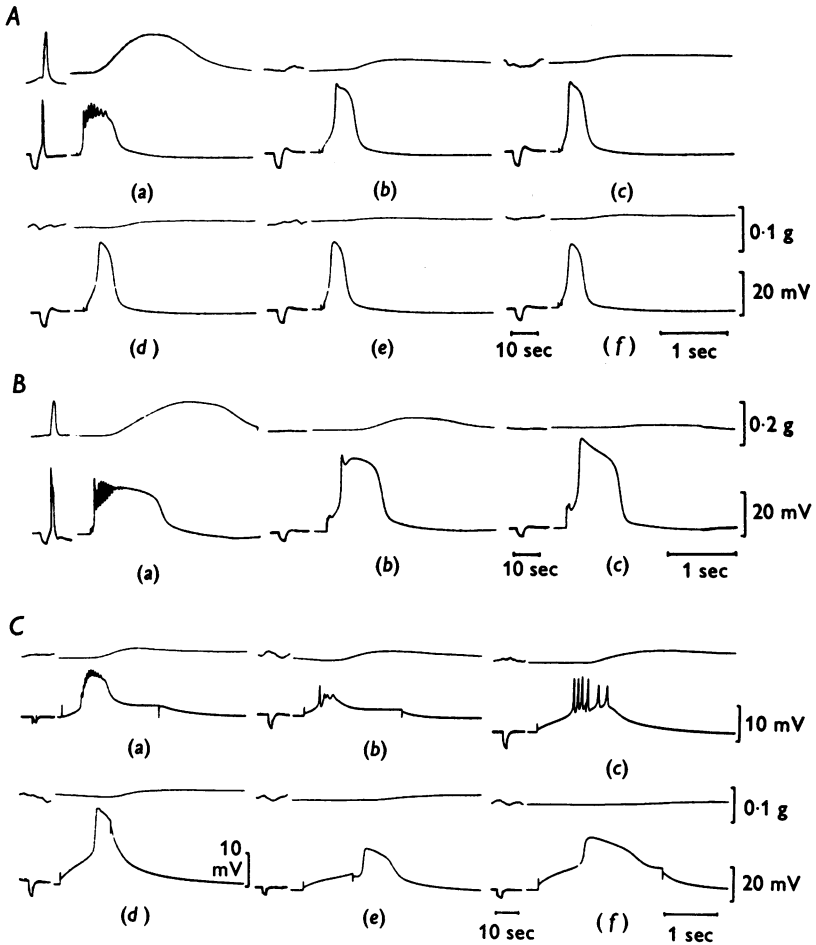

Fig. 4. *A*, the effect of Mn^{2+} (2 mM) on the action potential with poorly developed and *B*, pronounced plateau, and *C*, following Na-removal (sucrose). *A*, (*a*) in normal Locke solution; (*b–f*) 1, 2, 5, 10 and 20 min in 2 mM-Mn^{2+}. The current intensity for initiation of an action potential was increased to one-and-a-half-times in (*b*) and (*c*), to double in (*d*), and two-and-a-half-times in (*e*) and (*f*), relative to (*a*). In (*d–f*) the sensitivity for recording of contractions was increased twice compared with (*a–c*). *B*, (*a*) in normal Locke solution; (*b*) and (*c*) 1 and 5 min in Mn^{2+} (2 mM). In (*b*) and (*c*) the current intensity needed to trigger an action potential was increased to three and six-times that in normal conditions (*a*). *C*, effect of Na-removal and adding 2 mM-$MnCl_2$ to the Na-free (sucrose) solution: (*a*) in normal Locke solution; (*b*) and (*c*) 5 and 20 min after Na-removal; (*d–f*) 1, 4 and 12 min after the addition of 2 mM-$MnCl_2$ to Na-free solution. In (*e*) and (*f*) the gain was reduced to half.

The effect of Mn^{2+}

The possible role of Ca^{2+} ions in the generation of the action potential and of the contraction was studied indirectly by observing the action of Mn^{2+} ions. The experiments showed that 2 mM-$MnCl_2$ raised the threshold for initiation of the action potential approximately three to four-times, abolished the spike component and abruptly reduced the contraction in spite of a considerable increase of the amplitude of the remaining part of the action potentials. The duration at half-amplitude of the remaining part was however always less than that of the normal action potential. All the changes mentioned took place within the first few minutes after applying Mn^{2+} (Fig. 4*A*,*B*). Subsequently, there was no further change of the shape and amplitude of the action potential though the phasic contraction became progressively smaller (Fig. 4*A*,*B*). It is interesting to note that Mn^{2+} did not markedly affect the resting potential and the membrane resistance. The changes of the action potential and contraction produced by Mn^{2+} ions were easily reversible even after prolonged exposure to Mn^{2+}.

In another set of experiments the Mn^{2+} effect was investigated in Na-free Locke solution (sucrose substitution). If the spike component of the action potential is generated by Ca^{2+} entry, one would expect it to be depressed by Mn^{2+}. However, when 2 mM-Mn^{2+} was added to Na-free solution (Fig. 4*C*) both the amplitude and especially the duration of the action potential were increased, as in the presence of Na^+ (see Fig. 4*A* and *B*). The phasic contraction abruptly weakened and the threshold for muscle excitation was considerably increased. No significant changes in the resting potential and in the membrane resistance were observed.

The effect of verapamil

This Ca-antagonist is thought to act by blocking the Ca^{2+} inward current (Fleckenstein, Döring & Kammermeier, 1968; Fleckenstein, Grün, Tritthardt & Byon, 1971; Golenhofen & Lammel, 1972). It was found, as shown in Fig. 5, that verapamil at (10^{-5} M) increased the amplitude of the action potential within the first minutes but reduced the number of additional spikes as well as the duration of the plateau (Fig. 5*A*–*D*). Simultaneously, the amplitude of contraction gradually decreased. Membrane potential and membrane resistance were not substantially changed. Following the first 5 min of the verapamil action, the amplitude of the action potential declined to less than the initial size, the duration decreased to a greater extent and after 35 min only two spikes were observed. At this time the membrane was depolarized by 5–6 mV and the electrotonic potentials decreased by half (Fig. 5*F*).

In some experiments, after about 30 min exposure to verapamil, $MnCl_2$

was added to the solution in concentrations of 0·5–2 mM. As seen in Fig. 5*G–I*, it resulted in a considerable increase in the threshold of excitation, a gradual suppression of spikes and an increase in amplitude of the remaining part of the action potential. As a result, the spikes acquired a simple shape (Fig. 5*I*) similar to that seen when Mn^{2+} was added to normal Locke solution without verapamil (Fig. 4*A*). At the same time the contractions were abolished. The addition of Mn^{2+} to Locke solution containing verapamil had no significant effect on the resting potential and the membrane resistance.

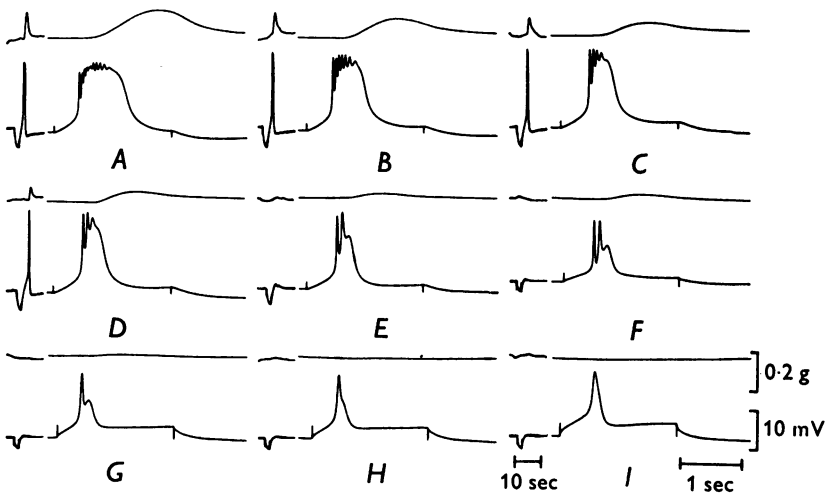

Fig. 5. The effect of verapamil, followed by Mn^{2+}. *A*, in normal Locke solution; *B–F*, 1, 2, 3, 5 and 35 min in verapamil (10^{-5} M); *G–I*, 1, 2 and 10 min after adding 0·5 mM-$MnCl_2$ to the solution containing verapamil. In *G–I* the current intensity required to trigger an action potential was increased to twice that of *A–F*.

The effect of K-free solution and ouabain

The main purpose of these experiments was to reduce the Na gradient across the membrane by increasing the intracellular Na^+ concentration due to inhibition of the Na–K pump. The record in Fig. 6*A* illustrates the tissue response at the end of the second hour of exposure to K-free Locke solution. By this time the fast and slow components of the action potential were less pronounced and the phasic contraction was prolonged. After 4 hr exposure to K-free solution, the duration of the action potential was decreased, mainly due to the shortening of the plateau, whereas the initial fast component was a spike (Fig. 6*B*). The amplitude of the associated phasic contraction was reduced, and its duration remained

prolonged. Thus prolonged incubation in K-free solution produced similar changes as Na-free solution.

The addition of 5·9 mM-KCl to the K-free Locke solution led to gradual recovery of the initial repetitive spike component, the subsequent plateau and the contraction (Fig. 6*D–F*), i.e. the action potential and contraction acquired the same shape and size which they have in normal conditions.

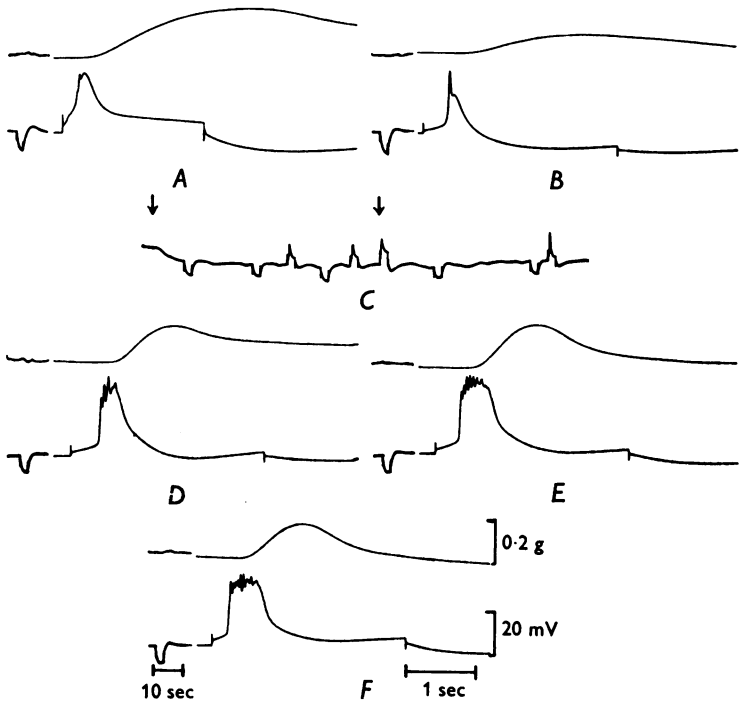

Fig. 6. Effect of removal and re-admission of K. *A*, the preparation had been incubated in K-free solution for 120 min and in *B*, for 240 min. *C*, shows hyperpolarization and reduction of electrotonic potential (on slow time base) within the first minute after re-admitting 5·9 mM-K (first arrow) to the K-free solution. The second arrow marks the increase in intensity of depolarizing current by two. *D–F*, recovery in normal Krebs solution 7, 10 and 20 min after *C*.

The addition of 5·6 mM-KCl to K-free solution also caused a hyperpolarization which attained its maximum by the end of the first minute. By the onset of the second minute after admission of normal solution, the hyperpolarization was accompanied by a considerable decrease in the size of the electrotonic potential, i.e. a decrease in membrane resistance. The threshold of excitation was raised (Fig. 6*B,C*). Subsequently, gradual recovery was observed (Fig. 6*D–F*) but it should be noted that 10 and

even 20 min after readmission of normal solution the size of the electrotonic potential was not restored to the value it had had before the addition of KCl to the K-free solution.

An increase in after-hyperpolarization was observed in K-free solution. This was studied by applying anodal pulses and observing the off spike. The experiments showed that the after-hyperpolarization was accompanied by a decrease of the electrotonic potential, i.e. a decrease in membrane resistance, the degree of which depended on the phase of the after-hyperpolarization (Fig. 7).

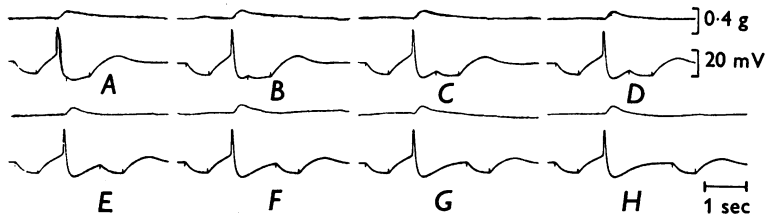

Fig. 7. Decrease of the electrotonic potential during the after-hyperpolarization observed after 180 min exposure to K-free Locke solution. In every record, the anelectrotonic potential with the off-spike was recorded and, for comparison, again at various stages of the after-hyperpolarization following the spike.

An investigation of the action of ouabain was carried out since ouabain, by inhibiting the Na–K pump, would also increase the intracellular Na^+ concentration, thereby reducing the Na gradient across the membrane and might cause effects similar to those observed by removal of external Na. As shown in Fig. 8*A*, the amplitude and duration of the action potential and of the contraction gradually decreased in the presence of ouabain (10^{-4} M). After 40 min the amplitude of the action potential was halved and the plateau was abolished (Fig. 8*A* (*a–f*)).

Ouabain produced a number of additional changes: a gradually developing depolarization reached a maximum after 30–40 min. At the beginning of this depolarization the size of the electrotonic potential increased, then diminished to the initial value and later decreased to about half of the original size. Ouabain also caused an increase in after-hyperpolarization during which the membrane resistance was decreased.

Assuming that during prolonged exposure to ouabain the remaining part of the action potential might be mainly generated by Ca^{2+} ions, it would be expected to be suppressed by Mn^{2+} ions. However, this was not so. The results of a typical experiment are presented in Fig. 8*B*. Exposure to ouabain for 1 hr decreased the amplitude of the action potential, almost abolished the plateau and decreased the contraction (Fig. 8*B* (*a–b*)). The

subsequent addition of 2 mM-$MnCl_2$ to the solution containing ouabain did not lead to a further depression but to an increase in the amplitude and duration of the action potential, although the associated contraction was markedly decreased (Fig. 8*B*(*c*)). Thus, in the presence of ouabain, the Mn^{2+} action is to some extent similar to its action in Na-free solution (Fig. 4*C*).

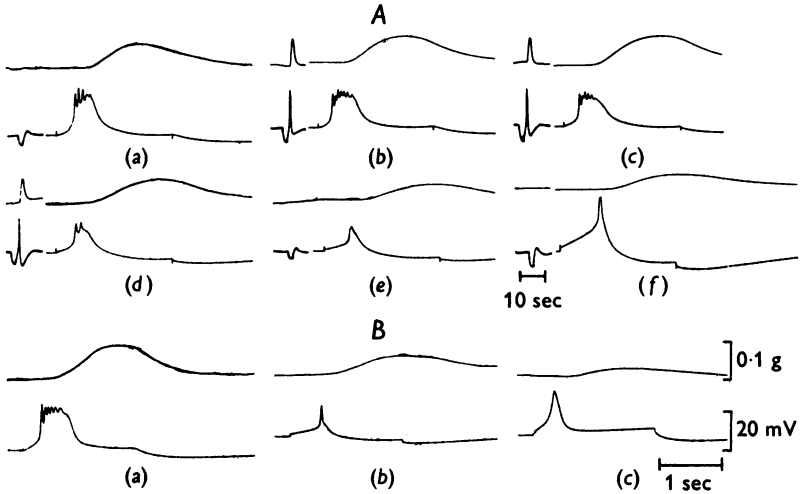

Fig. 8. *A*, effect of ouabain: (*a*) in normal Locke solution; (*b–f*) after 1, 10, 20, 30 and 40 min exposure to ouabain (10^{-4} M). In (*f*) the amplification was increased by 2. *B*, effects of ouabain and of Mn^{2+} in the presence of ouabain: (*a*) in normal Locke solution; (*b*) after 60 min exposure to ouabain (10^{-4} M); (*c*) 5 min after adding 2 mM-Mn^{2+} to the solution containing ouabain.

DISCUSSION

The observation that TEA increased the amplitude and duration of both the initial fast and the subsequent slow component of the action potential is consistent with the view that TEA specifically blocks the potential-dependent K conductance of the ureter smooth muscle membrane. Voltage-clamp experiments (V. A. Bury and M. F. Shuba, unpublished) have shown that TEA blocks the delayed K outward current, which consists of an initial fast and a subsequent slow component. The fast component of the outward current has a comparatively large amplitude, it starts almost simultaneously with the fast inward current but it rises more slowly. In the concentration used, TEA had no effect on membrane potential or membrane resistance that could account for the changes in the height of the action potential.

The reason for the suppression by TEA of additional spikes on the

plateau may be the considerable and long-lasting depolarization during the development of the plateau that leads to inactivation of the conductance of fast channels responsible for the appearance of repetitive spikes. This may also explain the gradual disappearance of additional spikes in normal conditions. It should be noted that TEA also increases the amplitude and duration of the action potential in other smooth muscles (Shuba, 1967, 1969; Ito, Kuriyama & Sakamoto, 1970; Vassort, 1974), and that this effect has been shown to be connected with the comparatively early activation of a delayed outward K current, which is blocked by TEA.

In the absence of Na^+, the duration of the action potential is decreased, mainly due to the shortening of the plateau. This observation is consistent with the suggestion that the plateau is chiefly due to the opening of slow Na channels. In addition, there occurs, in the absence of Na, a marked increase in membrane resistance and some hyperpolarization, probably due to a relatively large Na and Cl permeability of the smooth muscle cell membrane in normal conditions (Shuba, 1962, 1965, 1969; Kuriyama, 1970).

The study of the effects caused by exposure to K-free solution and ouabain yielded information concerning not only the role of Na^+ in the generation of the plateau but also concerning the Na–K pump and passive ionic membrane permeability. In Na-loaded muscle, the shortening of the action potential, mainly by shortening of the plateau, as well as the decreased amplitude and increased duration of the contraction are very like those caused by Na-free solution.

Casteels, Droogmans & Hendrickx (1971*a b*) have shown that in taenia coli, after 180–200 min exposure to K-free solution, the external and intracellular Na concentrations were equal, the intracellular K concentration being reduced to about 10 mM. The resting potential was decreased though the membrane resistance was within the initial range. The present results show that in the ureter after this time the values for the resting potential and action potential were similar to those obtained in normal solution. At first, when K^+ was re-admitted following exposure to K^+-free solution, a large but transient hyperpolarization was observed accompanied by a decrease in membrane resistance and reduced excitability of muscle cells. These changes are similar to those observed in guinea-pig taenia coli (Tomita & Yamamoto, 1971) and longitudinal muscle of the ileum (Bolton, 1973). Therefore, this hyperpolarization may be not only due to activation of the electrogenic Na pump but partly due to an increase in the K permeability.

The marked depolarization of the ureter by ouabain also can not be entirely accounted for by inhibition of the electrogenic Na pump since, like K-free solution, ouabain causes at first an increase in membrane resistance

and only later a decrease. It is therefore possible that the initial depolarization is mainly caused by a decrease in the K permeability.

The most characteristic changes caused by Mn^{2+} in the ureter are the following: reversible depression of the fast spikes; considerable increase in the amplitude of the remaining slow component of the action potential; abrupt lowering of excitability and abolition of contraction. The depression of fast spikes by Mn^{2+} ions is consistent with the suggestion that they are the result of Ca^{2+} entry into the muscle cells.

The increase in the amplitude of the remaining slow component of the action potential may be connected, firstly, with the fact that, under normal conditions, the slow inward current is partly compensated by an outward K current and that the outward K current is partly blocked by Mn^{2+} (V. Bury, unpublished). In the present study, Mn^{2+} ions increased the slow component of the action potential to a lesser extent in the presence of TEA than in normal conditions. Secondly, it is possible that in normal conditions the slow channels participating in the formation of the plateau are partly inactivated by depolarization of the membrane caused by the initial fast spikes.

The most unexpected results were obtained in the investigation of Mn^{2+} effects in Na-free solution, when the amplitude and duration of the action potential were not depressed but considerably increased while the phasic contraction was abolished. It is unlikely that the action potential was generated by Na^+ remaining in the intercellular spaces, because the effect was observed after incubation in Na-free solution for as long as 60–90 min. Moreover in Na-free solution containing Mn^{2+}, the amplitude of the action potential is linearly related to the log. of the extracellular Ca concentration, log $[Ca^{2+}]_o$, and is abolished in zero $[Ca^{2+}]_o$ (M. F. Shuba, unpublished). Therefore, in contrast to cardiac muscle (Ochi, 1975), the action potential of the ureter, in Na-free solution containing Mn^{2+} may not be due to Mn^{2+} but to Ca^{2+} entry through the slow Na-channels. Similarly, this may explain the appearance of action potentials after addition of Mn^{2+} to the solution following prolonged treatment with ouabain. It seems likely that also in normal conditions Ca^{2+} ions can pass through the slow Na-channels but may be insufficient to initiate a visible contraction.

The study of the action of verapamil was not consistent with reports that this substance blocks specifically only the spike component of the action potential (Golenhofen & Lammel, 1972). Following an initial increase, the amplitude of both spikes and plateau gradually declined and this coincided with the development of a considerable depolarization which may have been the main cause for the decrease of the action potential.

In normal conditions the ureter muscle possesses no tone. Hyperpolarization of the membrane by constant electric current and subthreshold

depolarization does not produce relaxation or contraction. A contraction appears only in response to an action potential.

The individual phasic contraction seems to be triggered by the initial fast component, while the amplitude and duration of the contraction are related to the amplitude and duration of the plateau which, by itself, may be unable to trigger a contraction. This requires further detailed investigation.

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